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## Breeding habits and associated phenomena in some Indian bats

### Part II—*Rhinolophus rouxi* (Temminck)—Rhinolophidae<sup>1</sup>

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*Rhinolophus rouxi* in Western Ghats near Khandala, Maharashtra State, has a sharply defined breeding season. The females in the colony undergo copulation some time during the last week of December and the early development of the egg is slow. There is a delayed implantation of the blastocyst. Each female carries a single conceptus invariably in the right uterine cornu. After a gestation period of about 150 to 160 days delivery takes place during the last week of May or early in June. This species does not attain sexual maturity in the year of birth. There is an uneven sex ratio with the females outnumbering the males in the adult, while during the juvenile stage the males outnumber the females.

#### INTRODUCTION

Detailed reviews of the earlier work on the breeding habits of microchiropteran bats have been given by several authors (Baker & Bird 1936; Wimsatt 1942; Gopalakrishna 1947, 1955; Madhavan 1971). From these reviews and from a perusal of the earlier literature on the subject it is evident that there are wide

variations in the breeding pattern of the microchiropteran bats and that different species inhabiting the same locality differ in their breeding habits.

The family Rhinolophidae has a wide geographic distribution and is represented in Europe, Africa, Asia and Australia. All rhinolophids so far studied appear to have a strict breeding periodicity, but there are fundamental differences between those living in cold climates and those in warmer regions.

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In the European rhinolophids (Fries 1879; Rollinat & Trouessart 1897; Courrier 1924; Matthews 1937) after copulation, which takes place in autumn, the inseminated sperms are stored in a ventrally located pocket of the vagina during winter, when the animals go into hibernation, and effectively fertilise the ova which are released in the following spring when the animals wake from hibernation. After copulation the vaginal canal is blocked by a plug which prevents further copulation. The scanty information which is available regarding the reproductive processes of Indian rhinolophids indicates that, while the periodicity of breeding is strict, there is nothing comparable to the protracted storage of inseminated sperms as noted in the European species (Ramakrishna Iyer 1951; Brosset 1962; Srinivasan *et al.* 1973). Further, there does not seem to be agreement among the authors regarding several aspects of breeding biology of Indian rhinolophids. *Rhinolophus rouxi*, collected from a restricted mountainous region of western ghats, has been chosen for detailed study not only because there is no work on this species from this locality, and this species appears to exhibit different breeding behaviour in different regions (Ramakrishna Iyer 1951; Brosset 1962; Srinivasan *et al.* 1973), and also because it exhibits certain extraordinary features which have not been observed in any Indian bat so far.

#### MATERIAL AND METHODS

The specimens of *Rhinolophus rouxi* were collected at random from three railway tunnels (Nos. 11, 12 and 13) near Khandala in western ghats, Maharashtra State, India. This region receives a heavy rain fall during June-September and has luxuriant vegetation. The specimens were collected at intervals of three to four weeks commencing from 7th October

1970 to 25th December 1972 such that every calendar month is represented by one collection or more. During the breeding season the specimens were collected at more frequent intervals with a view to obtaining closely graded development stages. Altogether 406 specimens were examined for the present work.

The specimens were killed by chloroform soon after capture and their body weight recorded. The characters of the external genitalia, the conditions of the mammary and the pubic teats in the females, and the position of the testis and the condition of the penis in the males were also recorded. The genital organs and the accessory reproductive structures were dissected out and fixed in various fixatives such as Bouin's fluid, neutral formalin and Rossman's fluid. After dehydrating them by passing through graded series of ethanol and clearing them in xylol, the gonads and the accessory organs were embedded in paraffin and sectioned at a thickness of 8  $\mu$ . The sections were stained with Ehrlich's haematoxylin, counterstained with eosin and mounted in DPX mountant.

The genital organs presented almost the same condition on a given calendar date in the two years when the collections were made. Hence, in the following descriptions, only the date and the month are mentioned, where pertinent, except where the mention of the year has a special significance.

A detailed collection diary incorporating the description of each specimen was maintained. Table 1 gives the summary of the collection diary and table 2 gives the month-wise collection of the specimens.

#### OBSERVATIONS AND DISCUSSION

##### 1. General remarks on *Rhinolophus rouxi*

The specimens live inside excavations in the walls of the railway tunnels in small

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TABLE 1

SUMMARY OF COLLECTION DIARY

| Date       | Males              |      |                |                    |      |            | Females   |              |          |    | Grand total |           |                  |
|------------|--------------------|------|----------------|--------------------|------|------------|-----------|--------------|----------|----|-------------|-----------|------------------|
|            | Immature           |      | Adult          | Immature           |      | Adult      |           | Non-pregnant | Pregnant |    |             | Lactating | Total of females |
|            | Attached to mother | Free | Total of males | Attached to mother | Free | Right horn | Left horn |              |          |    |             |           |                  |
| 1          | 2                  | 3    | 4              | 5                  | 6    | 7          | 8         | 9            | 10       | 11 | 12          | 13        |                  |
| 7-i-71     | —                  | 2    | 5              | 7                  | —    | —          | —         | 3            | —        | —  | 3           | 10        |                  |
| 9-i-71     | —                  | 2    | —              | 2                  | —    | 2          | —         | —            | —        | —  | 2           | 4         |                  |
| 11-i-71    | —                  | 2    | —              | 2                  | —    | 1          | —         | —            | —        | —  | 1           | 3         |                  |
| 14-i-71    | —                  | 4    | 3              | 7                  | —    | —          | —         | 1            | —        | —  | 1           | 8         |                  |
| 18-i-71    | —                  | 1    | —              | 1                  | —    | 1          | —         | —            | —        | —  | 1           | 2         |                  |
| 21-i-71    | —                  | 1    | 2              | 3                  | —    | —          | —         | —            | —        | —  | —           | 3         |                  |
| 23-i-71    | —                  | —    | 1              | 1                  | —    | —          | —         | 1            | —        | —  | 1           | 2         |                  |
| 26-i-71    | —                  | 1    | —              | 1                  | —    | —          | —         | —            | —        | —  | —           | 1         |                  |
| 1-ii-71    | —                  | —    | 1              | 1                  | —    | —          | —         | 9            | —        | —  | 9           | 10        |                  |
| 4-ii-71    | —                  | —    | 2              | 2                  | —    | 1          | —         | 11           | —        | —  | 12          | 14        |                  |
| 11-ii-71   | —                  | —    | 1              | 1                  | —    | 1          | —         | —            | —        | —  | 1           | 2         |                  |
| 12-ii-71   | —                  | 1    | —              | 1                  | —    | 2          | —         | 1            | —        | —  | 3           | 4         |                  |
| 19-ii-71   | —                  | 3    | 2              | 5                  | —    | —          | —         | —            | —        | —  | —           | 5         |                  |
| 23-ii-71   | —                  | 2    | 3              | 5                  | —    | —          | —         | 2            | —        | —  | 2           | 7         |                  |
| 26-ii-71   | —                  | —    | 16             | 16                 | —    | 2          | —         | 1            | —        | —  | 3           | 19        |                  |
| 16-iii-71  | —                  | 2    | 4              | 6                  | —    | 2          | —         | 15           | —        | —  | 17          | 23        |                  |
| 23-iii-71  | —                  | —    | 8              | 8                  | —    | 1          | —         | 11           | —        | —  | 12          | 20        |                  |
| 29-iii-72  | —                  | —    | 2              | 2                  | —    | 2          | —         | 1            | —        | —  | 3           | 5         |                  |
| 31-iii-72  | —                  | 1    | 1              | 2                  | —    | 1          | —         | 4            | —        | —  | 5           | 7         |                  |
| 1-iv-71    | —                  | —    | 4              | 4                  | —    | —          | —         | 9            | —        | —  | 9           | 13        |                  |
| 21-iv-71   | —                  | 2    | 1              | 3                  | —    | —          | —         | 2            | —        | —  | 2           | 5         |                  |
| 26-iv-72   | —                  | 1    | 7              | 8                  | —    | —          | —         | 10           | —        | —  | 10          | 18        |                  |
| 21-v-71    | —                  | —    | 3              | 3                  | —    | —          | —         | 7            | —        | —  | 7           | 10        |                  |
| 26-v-72    | —                  | —    | 2              | 2                  | —    | —          | —         | —            | —        | —  | —           | 2         |                  |
| 28-v-72    | —                  | —    | 7              | 7                  | —    | —          | —         | —            | —        | —  | —           | 7         |                  |
| 1-vi-72    | —                  | —    | 13             | 13                 | —    | —          | —         | —            | —        | —  | —           | 13        |                  |
| 14-vi-71   | —                  | 2    | 5              | 7                  | —    | —          | —         | —            | —        | 4  | 4           | 11        |                  |
| 14-vii-71  | —                  | 1    | 4              | 5                  | —    | 1          | —         | —            | —        | 23 | 24          | 29        |                  |
| 29-vii-72  | —                  | 1    | 16             | 17                 | —    | —          | —         | —            | —        | 7  | 7           | 24        |                  |
| 1-viii-71  | —                  | —    | 1              | 1                  | —    | 1          | —         | —            | —        | 8  | 9           | 10        |                  |
| 22-viii-71 | —                  | —    | —              | —                  | —    | 1          | —         | —            | —        | —  | 1           | 1         |                  |
| 27-viii-72 | —                  | —    | 3              | 3                  | —    | 2          | 17        | —            | —        | —  | 19          | 22        |                  |
| 17-ix-72   | —                  | —    | 3              | 3                  | —    | —          | 2         | —            | —        | —  | 2           | 5         |                  |
| 30-ix-71   | —                  | 2    | 5              | 7                  | —    | 1          | 4         | —            | —        | —  | 5           | 12        |                  |
| 7-x-70     | —                  | —    | 1              | 1                  | —    | —          | 27        | —            | —        | —  | 27          | 28        |                  |
| 22-x-71    | —                  | 1    | 2              | 3                  | —    | —          | 1         | —            | —        | —  | 1           | 4         |                  |
| 11-xi-70   | —                  | 2    | 4              | 6                  | —    | 1          | 16        | —            | —        | —  | 17          | 23        |                  |
| 24-xi-71   | —                  | 1    | 2              | 3                  | —    | —          | 1         | —            | —        | —  | 1           | 4         |                  |
| 13-xii-70  | —                  | 3    | 7              | 10                 | —    | 3          | 3         | —            | —        | —  | 6           | 16        |                  |

TABLE 2  
MONTHWISE COLLECTION OF SPECIMENS

| Month       | Male | Female | Total |
|-------------|------|--------|-------|
| Jan.        | 24   | 9      | 33    |
| Feb.        | 31   | 30     | 61    |
| Mar.        | 18   | 37     | 55    |
| Apr.        | 15   | 21     | 36    |
| May         | 12   | 7      | 19    |
| June        | 20   | 4      | 24    |
| July        | 22   | 31     | 53    |
| Aug.        | 4    | 29     | 33    |
| Sep.        | 10   | 7      | 17    |
| Oct.        | 4    | 28     | 32    |
| Nov.        | 9    | 18     | 27    |
| Dec.        | 10   | 6      | 16    |
| Grand total | 179  | 227    | 406   |

groups of 10 to 50 specimens. There may be several such groups within the same tunnel. During the non-breeding season there is a certain degree of segregation so that the adult males and females live in separate groups within the same tunnel, while during the breeding season they live together. Immature ones of both the sexes, however, occur in all the groups in all the seasons.

The fur on the body of *Rhinolophus rouxi* is usually black but a few specimens had brown coloured fur. Andersen (1917) noted that the change of the fur colour is normal for *Rhinolophus rouxi* and that it is related to moulting, which this species undergoes twice a year, once in spring (about May) and a second time in autumn (about October).

Although the tunnels from which these bats were collected were very busy railway tunnels, the specimens do not appear to be disturbed by the constant movement of trains, but any unusual sound or the approach of human beings near the groups appears to excite the specimens which flutter around and even fly out of the roost.

There were very few specimens in their normal roosts during the period from the middle of December to the first week of January when copulation takes place. Apparently, they appear to migrate elsewhere and return soon after the commencement of pregnancy.

This species does not undergo true hibernation as the European rhinolophids do, but they experience a slight degree of torpidity during July and August when there is incessant rain. Brosset (1962), however, mentioned that there is a definite hibernation in August in this species.

The adult females leave their normal roosts and migrate elsewhere just before delivering their young and return to their roosts only after the young are weaned. Hence, during this period the tunnels contained mostly males and immature females.

## 2. The female reproductive organs

The general construction of the female genitalia of *Rhinolophus rouxi* resembles that of most of the bats possessing bicornuate uterus. However, in the adult specimens the right uterine cornu is longer and noticeably thicker than the left. Each uterine cornu is about 1 cm long and the uterine cornua are symmetrically placed across the rectum and meet medially. The lumina of the uterine cornua become confluent, and a short cervical canal opens into the vaginal lumen. The vagina measures about 1 to 1.5 mm in length. Although the female genitalia are morphologically bilaterally symmetrical, only the right ovary releases a single ovum during each reproductive cycle and the conceptus is invariably carried in the right uterine cornu. Normally the follicles in the left ovary do not develop beyond the multilaminar stage, but in rare cases they may reach an early vesicular stage.



The mammary glands are on the ventrolateral sides of the thorax and their nipples are distinctly visible only in the parous animals. Pubic teats without mammary glands are present on each side near the groin. They are very small in immature animals, but get enlarged during the first pregnancy and remain as such throughout the rest of their life.

### 3. Breeding habits

Examination of the collection diary and table 1 reveals that pregnant specimens occurred only from about the first week of January to about the last week of May. This indicates that this species breeds once a year in a sharply defined season. Examination of the ovaries of the adult female specimens collected on 13th December revealed the presence of one large vesicular follicle in addition to numerous smaller follicles in the right ovary, and follicles at various stages of development up to the multilaminar condition in the left ovary. The uterine glands contained copious amount of secretion and the uterine lumen had some amount of cell debris. The vagina contained desquamated cornified cells. Evidently the animals were approaching oestrus. Neither copulation nor ovulation had occurred in these specimens. A late uterine morula was present in the right uterine cornu of adult females collected on the 7th January 1971, 14th January 1971 and 23rd January 1971. The morula in all these specimens was at nearly the same stage of development. The other females collected on these dates were immature. Each of the adult specimens collected between 1st February 1971 and 12th February 1971 contained an unimplanted blastocyst in the right uterine cornu. The adult females collected on 23rd February 1971 had each an early implanted blastocyst. Progressively advanced stages of pregnancy were noticed in the females collected during

the following weeks until 21st May 1971. Pregnant females each carrying a full term foetus, and which would have probably delivered within a week more, were collected on 21st May 1971. Further, every adult female in the colony was pregnant between 7th January 1971 and 21st May 1971, and the stage of development of the embryo was nearly the same in all the females collected on any given date. These facts lead to certain interesting conclusions:—

Since the adult specimens collected on 13th December had neither copulated nor did they have preovulatory follicles, and since all the adult females collected on 7th January had an uterine morula it is evident that fertilization must have occurred latest about 4 to 5 days before 7th January, 1971 in these animals. Secondly, there is a retarded development of the embryo during the early stages and a delayed implantation of the blastocyst, which remains free in the uterine lumen for a considerable time.

Between 21st May 1971 and 14th June 1971 neither pregnant females nor females in lactation were present in the railway tunnels which contained only males and immature females during this period. Many females in lactation were collected between the 14th of June and 1st of August, but there was no young at their breasts. These facts indicate that the females migrate elsewhere prior to parturition and that all the females in the colony deliver the young within the short span of two weeks. After parturition the females return to the roosts. The facts that the mothers do not carry the young ones at their breasts incessantly, and that the females were in full lactation until the 1st of August, indicate that the females give suck to the young ones in the roosts and do not normally carry the young ones at breast in flight as many

other bats do. The first free weaned young of the year was collected on the 1st of August.

From the foregoing account of the breeding habits of *Rhinolophus rouxi* the annual life of the females of this species can be recognized into the following periods:—

(1) The period of sexual quiescence from August to about the beginning of December.

(2) Copulation in a sharply defined period during the last week of December or the first week of January latest.

(3) Pregnancy involving retarded early development and delayed implantation from about the first week of January until about the last week of May.

(4) Parturition during the last week of May or early in June.

(5) Lactation from the last week of May or early in June to about the first week of August.

Since *Rhinolophus rouxi* has a sharply defined breeding season, it is relatively easy to determine the gestation period from the pregnancy record. From the stages of development of the morula in the three females collected on 7th January one could deduce that fertilization could have occurred about a week earlier. The last date on which the pregnant females were collected was 21st May when the females had each a full term foetus with pigmented skin and well developed patagia. The rhinarium and the median ridge above the nostrils of the foetuses had attained their full development. From these characters of the foetuses one could deduce that the young would have been delivered within another week. On the basis of these facts the duration of pregnancy in *Rhinolophus rouxi* should be about 150 to 160 days. This confirms the observation of Ramakrishna Iyer (1951), but it is at variance with those of Brosset (1962) and Srinivasan *et al.* (1973), who mention a gesta-

tion period of 60 to 70 days and three months respectively for this species.

#### 4. Age at maturity

As already mentioned, the young ones of *Rhinolophus rouxi* are delivered in a sharply restricted period between about the last week of May and the first week of June. The young animals grow rapidly during early life and reach a weight nearly equal to the weight of the adult when they are three to four months of age. After this age the young ones cannot be distinguished from the adults on the basis of the weight or the size of the body. However, sexual maturity or otherwise in the females can be determined on the basis of the size and nature of mammary nipples and pubic teats taken along with the pregnancy record. The mammary nipples and pubic teats are very insignificant in the non-parous females. After the first lactation they enlarge in size and remain as such throughout life. Hence, in all parous females they are prominent and large. Sexual maturity in males can be determined on the basis of the histology of the testis. The presence of a few immature females and males during the breeding season indicates that sexual maturity is not attained by either sex in the year of their birth in this bat. Since females are shown to become pregnant in January it is evident that they should be *at least* 19 months of age before attaining sexual maturity. Examination of the testes reveal that spermatogenesis commences late in September. Hence, the males take *at least* 16 months to reach sexual maturity. At least three categories of specimens of each sex can be recognized at the beginning of the breeding season— (a) animals which have bred at least once before, (b) animals which are in their first breeding season, and (c) animals which are sexually immature.

## 5. Sex ratio

In a total of 406 specimens of *Rhinolophus rouxi* collected at random over a period of two years, there were 227 females including 26 immature ones and 179 males including 38 immature ones. The fact that there is a certain degree of segregation of the two sexes may not warrant a definite conclusion regarding the sex ratio in this species. However, the

random collections made during two years shows an uneven sex ratio with the females outnumbering the males in the adult stage. In the juvenile stage, however, the males outnumber the females. It is likely that there is a greater mortality of the males than the females during juvenile life resulting in the uneven female-predominant sex ratio in the adults.

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